Data Harmonization Guidelines to Combine Multi-platform Genomic Data from Admixed Populations and Boost Power in Genome-Wide Association Studies

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Data harmonization involves combining data from multiple independent sources and processing the data to produce one uniform dataset. Merging separate genotypes or whole-genome sequencing datasets has been proposed as a strategy to increase the statistical power of association tests by increasing the effective sample size. However, data harmonization is not a widely adopted strategy due to the difficulties with merging data (including confounding produced by batch effects and population stratification). Detailed data harmonization protocols are scarce and are often conflicting. Moreover, data harmonization protocols that accommodate samples of admixed ancestry are practically non-existent. Existing data harmonization procedures must be modified to ensure the heterogeneous ancestry of admixed individuals is incorporated into additional downstream analyses without confounding results. Here, we propose a set of guidelines for merging multi-platform genetic data from admixed samples that can be adopted by any investigator with elementary bioinformatics experience. We have applied these guidelines to aggregate 1544 tuberculosis (TB) case-control samples from six separate in-house datasets and conducted a genome-wide association study (GWAS) of TB susceptibility. The GWAS performed on the merged dataset had improved power over analyzing the datasets individually and produced summary statistics free from bias introduced by batch effects and population stratification. © 2024 The Author(s). Current Protocols published by Wiley Periodicals LLC.

Basic Protocol 1: Processing separate datasets comprising array genotype data
Alternate Protocol 1: Processing separate datasets comprising array genotype and whole-genome sequencing data
Alternate Protocol 2: Performing imputation using a local reference panel
Basic Protocol 2: Merging separate datasets
Basic Protocol 3: Ancestry inference using ADMIXTURE and RFMix
Basic Protocol 4: Batch effect correction using pseudo-case-control comparisons

Keywords: ancestry inference • batch effects • data harmonization • genomic data • merging

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INTRODUCTION

Genetic association studies rely on large sample sizes to detect genetic variants (often single nucleotide polymorphisms, or SNPs) with differing allele frequencies between cases and controls. High-throughput sequencing technologies have enabled the quick and easy generation of large genomic datasets. However, consortia aiming to collect a large number of samples may need to genotype or sequence samples in “batches” spread out over time and, occasionally, at different facilities. In some instances, researchers may wish to merge existing data with new data, which may be generated using different genotype platforms. Eventually, the data produced from the separate batches will need to be combined before analyses (such as genome-wide association studies, or GWASs) can be performed. Data harmonization describes the efforts of combining raw data from multiple sources (under comparable conditions) and processing the data to produce a uniform dataset (Lee et al., 2018). Merging genome-wide genotype data from different study sites or different time points will increase the number of samples included in downstream analyses, thereby offering greater power to detect statistically significant associations. Although theoretically simple, merging multi-platform genotype data must be done with caution. Even if each separate dataset has undergone quality control (QC) independently, the merged dataset will require additional QC, as errors and confounding factors can be introduced at any point in the merging process.

A standard operating procedure (SOP) outlining the best practices for merging multi-platform genomic data would be a valuable resource to geneticists aiming to harmonize separate datasets. However, the complexity of merging separate genetic datasets is often overlooked, and details regarding data harmonization procedures are not often published. Existing publications that provide data harmonization guidelines are not comprehensive and require modification for populations with complex population structure. For instance, the electronic MEdical Records and GEnomics (eMERGE) network (Gottesman et al., 2013) published best practices for merging genome-wide genotyping data between 2011 and 2019. During the first phase (eMERGE-I), the network merged genotype data from two different genotyping centers to form one large dataset comprising 17,000 individuals (Zuvich et al., 2011). The eMERGE-I network developed a QC pipeline and published approaches to merge data successfully by ensuring uniform strand orientation, examining sample quality, assessing for population stratification, performing marker quality checks, and checking for batch effects. However, for the first phase of the network, the same genotype array was used at the two genotyping centers, which simplified the merging process. During the second phase of the eMERGE network (eMERGE-II), multiple different genotyping platforms were used to generate genome-wide genotype data for over 50,000 samples (Stanaway et al., 2019). To combine all the eMERGE-II datasets, imputation was necessary to infer the missing genotypes and increase the number of overlapping sites between disparate genotyping platforms. A related paper from 2019 outlines best practices for merging and analyzing datasets obtained from different genotype microarrays (Stanaway et al., 2019). However, the paper does not mention methods to control for batch effects. In 2022, Chen et al. published a data harmonization pipeline that merges case-control genotype data obtained from different array platforms. Their methods aimed to leverage the use of publicly available controls to increase the number of samples and improve the power of GWASs (Chen et al., 2022). Their pipeline contains four modules, which involve QC of individual array datasets, imputation, post-imputation QC, and re-imputation. Their pipeline also outlines steps for resolving batch effects between datasets genotyped on different array platforms. However, the article only includes a homogeneous group of samples representing individuals of European ancestry. The authors acknowledged that modifying their pipeline for admixed samples would be advantageous as human populations become more admixed with increasing migration and globalization.

Here, we present a comprehensive set of guidelines for combining multi-platform genetic data obtained from individuals with complex admixed ancestry. Basic Protocol 1 describes the QC and impxutation procedures required to prepare the separate datasets. Alternate Protocol 1 details special considerations for merging whole-genome sequencing (WGS) data with array genotype data. Alternate Protocol 2 describes the steps for conducting imputation on a local reference panel on a high-performance computer (HPC) cluster. Basic Protocol 2 depicts the process of merging separate datasets in detail for the non-expert investigator. Basic Protocol 3 describes the process of investigating population structure through cross-validation (CV) and conducting global ancestry inference with two popular ancestry inference tools: ADMIXTURE (Alexander & Lange, 2011) and RFMix (Maples et al., 2013). Finally, Basic Protocol 4 describes how to screen and correct for the presence of batch effects in the merged dataset. Basic Protocol 4 builds on the guidelines provided by Chen et al. (2022) but has been modified to accommodate admixed individuals with the inclusion of global ancestry proportions. In the Commentary, we describe our application of the protocols. We have applied our data harmonization strategy to merge six different TB case-control cohorts (including those assayed on different genotype arrays) comprising individuals from a complex, multi-way admixed South African population. A GWAS performed on the merged dataset had improved power over analyzing the datasets individually and produced summary statistics free from bias introduced by batch effects and population stratification. This data harmonization approach, although by no means comprehensive, is the first of its kind designed specifically for multi-way admixed individuals with complex genetic structure and thus fills a gap in the current body of literature.

PROCESSING SEPARATE DATASETS COMPRISING ARRAY GENOTYPE DATA

BASIC PROTOCOL 1

This protocol provides instructions for QC and imputation of the separate genotype datasets using PLINK binary (`.bed + .bim + .fam`) or VCF (`.vcf`) file formats as input files. First, we illustrate how to perform pre-imputation QC using PLINK v2.0 (Purcell et al., 2007) to remove samples and sites with poor quality. Second, we show how to prepare and upload QC'ed files for imputation by the Sanger Imputation Server (SIS) (McCarthy et al., 2016). Lastly, we show how to filter poorly imputed sites using a predefined imputation quality/INFO score threshold.

We recommend imputing individual datasets separately prior to merging. Only intersecting SNPs across all datasets should be merged to avoid high levels of missingness in the combined dataset. Because different genotype arrays vary in the number of SNPs assayed, the number of intersecting SNPs across arrays will be minimal when merging genotype data obtained from disparate platforms before imputation. Imputation performance on the merged dataset will subsequently be affected, as imputation performance depends on the number of genotyped markers matching the haplotype reference panels. Hence, imputing individual datasets separately before merging achieves the greatest number of intersecting SNPs across datasets and improves the quality of imputed genotypes.

Necessary Resources

Hardware

A computer with as much memory (≥8 GB) and computing power as possible or access to an HPC cluster. A computer running either a 64-bit Linux-based distribution (e.g., Ubuntu) or macOS is required. A reliable internet connection is also required to access the SIS.

Software

PLINK v2.0 (*https://www.cog-genomics.org/plink2/*)
VCFTOOLS v0.1.17 (*https://vcftools.github.io/index.html*)
BCFTOOLS v1.9 (*https://samtools.github.io/bcftools/howtos/install.html*)
Globus Connect Personal (*https://www.globus.org*)

Files

Array genotype data in PLINK binary or VCF format
GRCh37 or GRCH38 reference FASTA file (depending on the human assembly the genotype data is aligned to)

1. Perform genotype QC on individual datasets.

Standard genotype QC procedures typically remove SNPs and individuals with missing information, monomorphic sites (sites that have a minor allele frequency (MAF) ≤ $\frac{1}{2n}$, where n represents the number of individuals in the dataset), and SNPs deviating from Hardy-Weinberg equilibrium (HWE). Variants with a genotype call rate <95% and variants that deviated from the HWE p-value threshold of 0.00001 should be excluded. Individuals with average genotype call rates <90% should also be removed. Mitochondrial DNA and the Y-chromosome should be removed from the genotype data if imputing through the SIS, which only imputes autosomal and X-chromosome sites. These stringent QC thresholds were used in our study to ensure there were no incorrectly genotyped variants in the data that could influence downstream protocols but may be adjusted based on the specific requirements of independent studies.

PLINK can be used to perform genotype QC on files in PLINK binary file format. Marees et al. (2018) have published detailed genotype QC procedures using PLINK, and their tutorial should be consulted by readers who are unfamiliar with the PLINK software. Users should also become familiar with the Input Filtering subsection of the PLINK documentation webpage (https://www.cog-genomics.org/plink2/):

```
plink --bfile input_filename --geno 0.05 --mind 0.1 --hwe 0.00001 --maf --chr 1-22 --make-bed
--out output_filename
```

VCFTOOLS can be used to perform genotype QC on files in VCF file format:

```
vcftools --vcf input_filename --max-missing 0.95 --hwe 0.00001 --maf --max-alleles 2 --
recode --recode-INFO-all --out output_filename
```

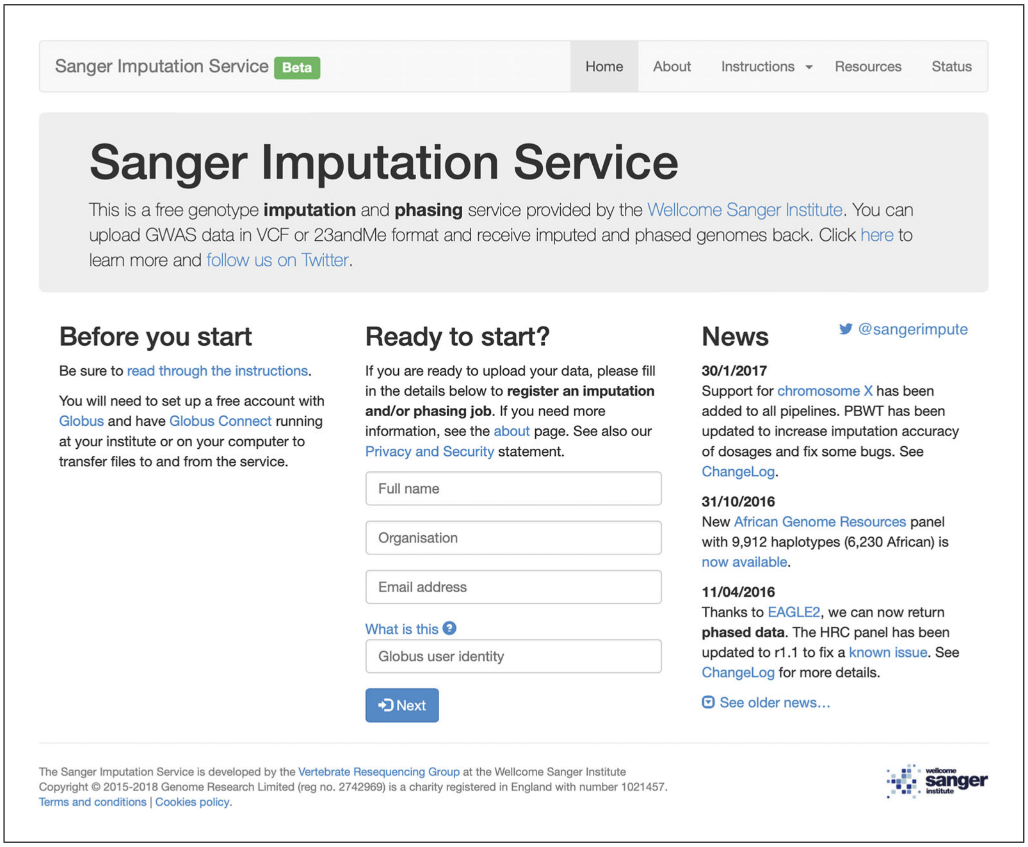
2. Convert the PLINK binary files to VCF file format before uploading to the SIS for imputation:

```
plink --bfile binary_fileset --recode vcf-iid --out vcf_file
```

3. Navigate to the SIS homepage to upload VCF file for imputation (*https://imputation.sanger.ac.uk*).

The SIS has instructions for uploading and downloading data using Globus (https://www.globus.org). Ensure the reference alleles match the reference genome (either GRCh37/hg19 or GRCh38) using BCFTools:

```
bcftools +fixref input_filename.vcf -- -f reference.fa -m top -Ov -o output_filename.vcf
```



Sanger Imputation Service

This is a free genotype **imputation** and **phasing** service provided by the Wellcome Sanger Institute. You can upload GWAS data in VCF or 23andMe format and receive imputed and phased genomes back. Click here to learn more and follow us on Twitter.

Before you start

Be sure to read through the instructions.

You will need to set up a free account with Globus and have Globus Connect running at your institute or on your computer to transfer files to and from the service.

Ready to start?

If you are ready to upload your data, please fill in the details below to **register an imputation and/or phasing job**. If you need more information, see the about page. See also our Privacy and Security statement.

Full name

Organisation

Email address

What is this ?

Globus user identity

Next

News

@sangerimpute

30/1/2017
Support for chromosome X has been added to all pipelines. PBWT has been updated to increase imputation accuracy of dosages and fix some bugs. See ChangeLog.

31/10/2016
New African Genome Resources panel with 9,912 haplotypes (6,230 African) is now available.

11/04/2016
Thanks to EAGLE2, we can now return **phased data**. The HRC panel has been updated to r1.1 to fix a known issue. See ChangeLog for more details.

See older news...

The Sanger Imputation Service is developed by the Vertebrate Resequencing Group at the Wellcome Sanger Institute
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Terms and conditions | Cookies policy.

Figure 1 Home page of the Sanger Imputation Server.

Users can select the imputation algorithm and reference panel from several options on the SIS. Users are also given an option to pre-phase their genotype data prior to imputation. Select an imputation method and reference panel appropriate for your study population and follow the instructions for uploading the array genotype VCF file to the SIS.

Imputation algorithm and reference panel options are available on the SIS (Fig. 1).

Our study used the SHAPEIT + PBWT pre-phasing and imputation algorithm with the African Genome Resource reference panel, as this imputation method was previously shown to produce the highest quality and accuracy in imputed data in our study population (Schurz et al., 2019). However, the choice of imputation algorithm and haplotype reference panel depends largely on the population structure of the study cohort. Numerous studies have explored imputation performance in different study populations. For example, imputation accuracy and quality using the 1000G Phase 3 reference panel in two- and three-way admixed populations has been evaluated by Nelson et al. (2016). Furthermore, imputation performance for populations with African ancestry has been extensively investigated using different combinations of imputation algorithms, reference panels, and imputation servers (Hancock et al., 2012; Roshyara et al., 2016; Sengupta et al., 2023). We recommend that users carefully consider the available imputation reference panels and test different combinations to determine the best algorithm and reference panel for their study population. It is important to consider that reference panels used for imputation possess individuals obtained from a similar population group to the cohort under study because haplotype structures vary across different population groups (Schurz et al., 2019). As imputation accuracy is dependent on the adequate matching of individual haplotypes to a reference, closely matching of reference and sample haplotypes is crucial to optimize the number of accurately imputed genotypes.

4. Download the imputed GZVCF files from the SIS (instructions can be found at *https://imputation.sanger.ac.uk/?instructions=1#downloadyourdata*).

The SIS returns per-chromosome VCF files. Concatenate per-chromosome imputed files using BCFTools to produce one VCF (containing all chromosomes and individuals):

```
bcftools concat *.impute.vcf.gz -Oz -o allChromosomes_impute.vcf.gz
```

Although the SIS conducts pre-phasing, the server does not return the phased output files derived by SHAPEIT2. Thus, users will need to re-phase the imputed data to obtain phased genotypes. Detailed steps for phasing using SHAPEIT2 are outlined in Basic Protocol 3.

5. Filter out poorly imputed genotypes to reduce uncertainty of downstream results.

The INFO score can be used to indicate the quality of imputed genotypes. The INFO score is a numerical value between 0 and 1, where values near 1 indicate a high degree of certainty that the imputed SNP is the correct genotype. The VCF files obtained from the SIS contain INFO scores for each imputed SNP. The INFO metric can be used to filter out poorly imputed SNPs using BCFTools. An appropriate INFO score threshold can be selected by the investigator (in this example, genotypes with INFO scores <0.8 are excluded).

```
bcftools filter -e ‘INFO/INFO<0.8’ input_filename.vcf.gz -Oz -o output_filename.vcf.gz
```

If INFO scores are not included in the VCF files generated by the imputation server, they can be generated from the posterior genotype probabilities using BCFTools:

```
bcftools +impute-info input_filename.vcf.gz -Oz -o output_filename.vcf.gz
```

ALTERNATE PROTOCOL 1

PROCESSING SEPARATE DATASETS COMPRISING ARRAY GENOTYPE AND WHOLE-GENOME SEQUENCING DATA

Merging WGS data with array genotype data requires special considerations. Genotype data obtained from different cohorts should only be combined using intersecting or common SNPs across all groups to avoid high rates of missing data in the final merged dataset. The high-coverage genotype data obtained from WGS has the ability to completely overlap with markers on any array. However, merging WGS and array data can be complicated by differences in genotyping technologies with a high potential for introducing batch effects. This alternate protocol, adapted from the GAWMerge protocol for combining array-based genotyping and WGS data (Mathur et al., 2022), provides instructions for preparing WGS data for merging with genotype data obtained from different array platforms.

Necessary Resources

Hardware

A computer with as much memory (≥8 GB) and computing power as possible or access to an HPC cluster. A computer running either a 64-bit Linux-based distribution (e.g., Ubuntu) or macOS is required.

Software

PLINK v2.0 (*https://www.cog-genomics.org/plink2/*)

Files

WGS data in VCF format

List of SNPs genotyped on one of the arrays to be merged. We suggest extracting a list of SNPs from the array with the greatest marker density. For example, in our study, we used a list of SNPs genotyped on the Illumina H3Africa array (the SNP IDs can be obtained from *https://chipinfo.h3abionet.org/browse*).

1. Generate the list of SNPs to be extracted from the WGS data.

The list should be in text format, with the rsIDs or position of the SNP in chromosome:basepair format (one SNP per line, no headers). Regardless of the method, ensure *that the corresponding reference genome used for genotype calling across datasets is consistent.*

2. Extract the SNP list from WGS data using PLINK and convert the output file to binary PLINK file format:

```
plink --vcf input_filename.vcf --extract SNP_list.txt --make-bed --out output_filename
```

3. Follow QC, imputation, and post-imputation QC procedures outlined in Basic Protocol 1, steps 2 to 5.

PERFORMING IMPUTATION USING A LOCAL REFERENCE PANEL

ALTERNATE PROTOCOL 2

In the event that researchers have a local/personalized reference panel for imputation or do not wish to use a remote imputation server, phasing and imputation can be performed by the researcher on an HPC cluster. This alternate protocol provides instructions for phasing and imputation using Eagle (Loh et al., 2016) and Minimac4 (Howie et al. 2012), respectively. This protocol follows from the genotype QC procedures outlined in Basic Protocol 1, steps 1 and 2.

Necessary Resources

Hardware

A computer with as much memory (≥8 GB) and computing power as possible or access to an HPC cluster. A computer running either a 64-bit Linux-based distribution (e.g., Ubuntu) or macOS is required.

Software

PLINK v2.0 (*https://www.cog-genomics.org/plink2/*)
Eagle v2.4.1 (phases genotypes prior to imputation: *https://alkesgroup.broadinstitute.org/Eagle/#x1-30002*)
Minimac4 (*https://github.com/statgen/Minimac4*)
BCFTOOLS v1.9 (*https://samtools.github.io/bcftools/howtos/install.html*)

Files

Reference genetic map files (included in the Eagle software download)
Phased reference genotypes in tabix-indexed VCF format

1. Split target and reference VCFs into individual chromosome files using PLINK:

```
for i in {1..22}; do plink --vcf TargetSamples.vcf --chr ${i} --recode vcf --out
TargetSamples _chr${i}; done

for i in {1..22}; do plink --vcf ReferenceSamples.vcf --chr ${i} --recode vcf --out
ReferenceSamples _chr${i}; done
```

2. Phase target per-chromosome VCFs using Eagle and the phased reference VCF:

```
for i in {1..22}; do Eagle_v2.4.1/eagle --noImpMissing --vcfOutFormat=z --vcfTarget
TargetSamples _chr${i}.vcf --geneticMapFile Eagle_v2.4.1/tables/genetic_map_hg38_
withX.txt.gz --outPrefix TargetSamples _chr${i}.phased --vcfRef ReferenceSamples_
chr${i}.vcf; done
```

3. Impute missing genotypes in phased target per-chromosome VCFs using Minimac4 and the phased reference VCF:

```
for i in {1..22}; do minimac4 --refHaps ReferenceSamples_chr${i}.vcf --haps
TargetSamples_chr${i}.phased.vcf.gz --allTypedSites --cpus 10 --prefix
TargetSamples_imputed_chr${i}; done
```

The per-chromosome imputed VCF files can then be concatenated using BCFTools to produce one VCF (containing all chromosomes and individuals).

```
bcftools concat TargetSamples_imputed_chr*.vcf -Oz -o

allChromosomes_impute.vcf.gz
```

BASIC PROTOCOL 2 MERGING SEPARATE DATASETS

Only intersecting or common SNPs across all separate datasets should be combined to avoid high rates of SNP missingness in the final merged dataset. This protocol describes the process of identifying and extracting intersecting SNPs from each genotype dataset, combining the separate datasets, and conducting post-merging QC procedures, such as removing duplicate or closely related individuals. In this protocol, KING software is used to identify closely related individuals. Users should familiarize themselves with the software user guide prior to beginning this protocol to understand the files generated by KING and the kinship coefficients used to estimate relatedness (*https://www.kingrelatedness.com/manual.shtml*).

Necessary Resources

Hardware

A computer with as much memory (≥8 GB) and computing power as possible or access to an HPC cluster. A computer running either a 64-bit Linux-based distribution (e.g., Ubuntu) or macOS is required.

Software

Java v1.8 (to run the Picard toolset; can be obtained from *https://www.oracle.com/java/technologies/downloads/*)
PLINK v2.0 (*https://www.cog-genomics.org/plink2/*)
KING v2.3.2 (*https://www.kingrelatedness.com/Download.shtml*)

Files

Reference sequence FASTA file (the target sequence for the required genomic build coordinates; can be obtained from *https://hgdownload2.soe.ucsc.edu/downloads.html#human*)
UCSU chain file to guide genomic coordinate conversion from one genome build to another (can be obtained from *http://hgdownload.soe.ucsc.edu/downloads.html#liftover*)

1. If required, convert genomic positions to the same coordinate system (e.g., hg19, hg38).

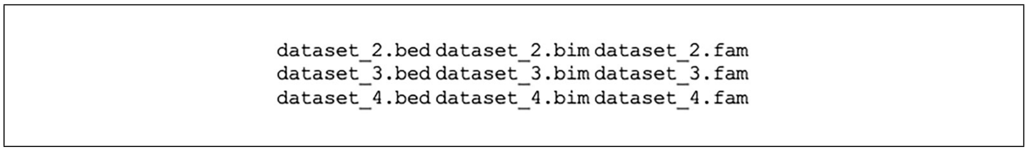
   The LiftoverVcf tool from GATK can be used to "lift over" genetic positions in a VCF from one genome build to another. The LiftoverVCF tool forms part of the Picard command-line tools, which are provided in a single executable `picard.jar` *file (which can be downloaded from https://github.com/broadinstitute/picard/releases/tag/3.1.1). Follow the instructions at https://broadinstitute.github.io/picard/ to download and install the Picard toolset.*

   Once the toolset has been installed, run the following commands to convert VCF genomic coordinates from one genome build to another (e.g., from hg19 to hg38):

   ```
   java -jar picard.jar LiftoverVcf \\
   I=input.vcf \\
   O=lifted_over.vcf \\
   CHAIN=b37tohg38.chain \\
   REJECT=rejected_variants.vcf \\
   R=reference_sequence.fasta
   ```

2. Remove poorly imputed genotypes (see Basic Protocol 1, step 5) and convert imputed VCF files to binary PLINK file format:

   ```
   plink --vcf filename.vcf --make-bed --out filename
   ```



```
dataset_2.bed dataset_2.bim dataset_2.fam
dataset_3.bed dataset_3.bim dataset_3.fam
dataset_4.bed dataset_4.bim dataset_4.fam
```

Figure 2 Example of the text file containing the list of files to be merged.

3. Screen for duplicate or closely related individuals within each separate dataset using KING software.

   Identical by descent (IBD) segments are rapidly inferred, and individuals in relationship pairs (such as parent/offspring or sibling pairs) are identified using KING:

   ```
   king -b input_filename.bed --ibdseg
   ```

   One individual from each relationship pair identified should be chosen to be removed from the dataset. The `remove_relatives.py` *script(developed as part of the PONDEROSA algorithm; https://github.com/williamscole/PONDEROSA/tree/master) compiles a list of unrelated individuals, given the* `.seg` *file generated by the above KING command and the* `.fam` *file from PLINK. For example, pairs of individuals who are less than second-degree relatives will be identified in the following command:*

   ```
   python remove_relatives.py None king.seg input_filename.fam 2nd
   ```

   Use PLINK and the output list of unrelated individuals to filter related individuals from the dataset:

   ```
   plink --bfile input_filename --keep unrelated_individuals.txt --make-bed -
   -outoutput_filename
   ```

   Repeat this procedure for each separate dataset.

4. Convert all SNP positions to chromosome:basepair format using PLINK:

   ```
   plink --bfile input_filename --set-all-var-ids @:# --make-bed --out output_filename
   ```

   Then, extract a list of SNPs in each dataset:

   ```
   awk '{print $2}' input_filename.bim | sort > SNP_list.txt
   ```

5. Compare the SNP lists from all datasets to identify common or intersecting sites.

   For example, here, we compare SNP list files from four separate datasets to identify intersecting SNPs across all datasets:

   ```
   comm -12 SNP_list_1.txt SNP_list_2.txt |
   comm -12 - SNP_list_3.txt |
   comm -12 - SNP_list_4.txt > Intersecting_SNPs_allDatasets.txt
   ```

6. Extract intersecting SNPs from each dataset:

   ```
   plink --bfile input_filename --extract Intersecting_SNPs_allDatasets.txt --make-bed --out
      output_filename
   ```

7. Use PLINK to merge the intersecting SNPs from each dataset.

   ```
   plink --bfile dataset_1 --merge-list Files_to_merge.txt --make-bed --out Merged_files
   ```

   If there are more than two datasets to merge, a text file with the `.bed + .bim + .fam` *files of each dataset must be provided (Fig. 2).*

   PLINK may return with an error that the files cannot be merged due to sites having multiple alleles or multiple chromosomes/positions. If strand errors are suspected, variants with flipped alleles can be corrected using PLINK (detailed steps of this procedure can be found on the PLINK documentation webpage: https://www.cog-genomics.org/plink/1.9/data#merge3). Alternatively, if problematic SNPs cannot be corrected by resolving strand flip errors, the residual offending SNPs can be removed (PLINK will automatically output a list of these SNPs with multiple alleles or allele mismatched if the initial merge failed).

```
plink --bfile dataset_1 --exclude Merged_files.missnp --make-bed --out
dataset_1_RemoveProblemSNPs

plink --bfile dataset_2 --exclude Merged_files.missnp --make-bed --out
dataset_2_RemoveProblemSNPs
```

Repeat this procedure for each dataset and attempt the merge again:

```
plink --bfile dataset_1_tmp --merge-list Files_to_merge.txt --make-bed --out Merged_files
```

8. After merging, apply additional QC steps to remove residual genotyping errors in SNPs and samples with low genotype call rates. Remove all individuals missing >10% genotypes, exclude markers with >5% missing data, and apply an HWE filter to controls (threshold < 0.00001):

```
plink --bfile Merged_files --geno 0.05 --mind 0.1 --hwe 0.00001 --make-bed --out
    Merged_files_QC
```

9. As duplicate or related individuals may exist across different datasets, identify IBD segments and individuals in relationship pairs (such as parent/offspring or sibling pairs) using KING:

```
king -b input_filename.bed --ibdseg
```

Choose one individual from each relationship pair identified to be removed from the dataset.

The `remove_relatives.py` script (developed as part of the PONDEROSA algorithm—https://github.com/williamscole/PONDEROSA/tree/master) compiles a list of unrelated individuals, given the `.seg` file generated by the above KING command and the `.fam` file from PLINK. For example, pairs of individuals who are less than second-degree relatives will be identified in the following command:

```
python remove_relatives.py None king.seg input_filename.fam 2nd
```

Use PLINK and the output list of unrelated individuals to filter related individuals from the dataset:

```
plink --bfile input_filename --keep unrelateds.txt --make-bed --out output_filename
```

Sample Data

For sample data, see a list of files to be merged in Figure 2.

BASIC PROTOCOL 3

ANCESTRY INFERENCE USING ADMIXTURE AND RFMIX

Population stratification (allele frequency differences between cases and controls due to differences in population structure) may confound association signals between genotype and phenotype (see Current Protocols article: Hellwege et al., 2017). Although controlling for population structure is a routine procedure in GWASs and other downstream analyses, it is particularly important when working with genotype data obtained from admixed individuals, whose ancestry is highly heterogeneous across the genome (Swart et al., 2021). Although principal components (PCs) are commonly included as covariables in GWASs to control for population structure, PCs cannot reliably deconvolute recent population structure present in complex, highly admixed populations (Elhaik, 2022). PC analysis (PCA) appropriately captures older population deviation (i.e., separation among continental population groups) (Patterson et al., 2010). However, more recent population structure (i.e., recent admixture among population clusters) cannot accurately be discerned from PCA (Petersen et al., 2013). Calculating contributing ancestral proportions is a superior method to PCA to account for population stratification in samples with recent admixture. This protocol provides instructions for estimating global ancestry proportions for individuals in the merged dataset generated by Basic Protocol 3. First, we illustrate how to produce a file of reference sample individuals and how to run the ADMIXTURE (Alexander & Lange, 2011) ancestry inference software. In this protocol, ADMIXTURE is run in an unsupervised manner to first determine the correct number of source populations contributing to the overall study population structure. Thereafter, ADMIXTURE is used to determine the global ancestry proportions of each sample. As always, users should familiarize themselves with the software user documentation prior to beginning this protocol (*https://dalexander.github.io/admixture/admixture-manual.pdf*). The second half of this protocol describes how to use RFMix to infer global ancestry proportions given the number of source populations determined in the first half of the protocol. Users should familiarize themselves with the RFMix user documentation (*https://github.com/slowkoni/rfmix/blob/master/MANUAL.md*) before beginning this protocol. Although the ADMIXTURE algorithm is perhaps the most common method of ancestry inference, RFMix (Maples et al., 2013) has been shown to outperform ADMIXTURE in determining global ancestry proportions in complex multi-way admixed populations (Uren et al., 2020). Additionally, Basic Protocol 4 requires RFMix output files. It is important to note that RFMix does not determine the correct number of contributing source populations (i.e., K). Thus, we recommend that researchers use ADMIXTURE to determine K, followed by RFMix to refine the global ancestry fractions. ADMIXTURE and RFMix have been routinely applied to complex multi-way admixed samples with success in our experience; thus, they are our software of choice for ancestry inference in populations with complex admixture patterns.

Necessary Resources

Hardware

A computer with as much memory (≥8 GB) and computing power as possible or access to an HPC cluster. A computer running either a 64-bit Linux-based distribution (e.g., Ubuntu) or macOS is required.

Software

PLINK v2.0 (*https://www.cog-genomics.org/plink2/*)
ADMIXTURE v1.3 (*https://dalexander.github.io/admixture/download.html*)
PONG v1.5 (*https://github.com/ramachandran-lab/pong/tree/master*)
SHAPEIT v2 (*https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html*)
VCFTOOLS v0.1.17 (*https://vcftools.github.io/index.html*)
RFMix v2 (*https://anaconda.org/bioconda/rfmix*)

Files

Reference sample individuals
Genetic map files
Sample map file

1. Prepare a reference file.

The reference file is one file comprising individuals from different populations that will serve as proxies for the ancestral/source populations of the target individual. The reference file can be compiled using individuals from public data repositories, such as the 1000 Genomes Project (1000GP) (https://www.internationalgenome.org). To download reference data from the 1000GP repository:

```
wget
ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/ALL.chr*.phase3_shapeit2_mvncall_
integrated_v5a.20130502.genotypes.vcf.gz
```

This will download per-chromosome files containing all individuals from the 1000GP phase 3 callsets.

2. Concatenate per-chromosome imputed files using BCFTools to produce one VCF (containing all chromosomes and individuals):

```
bcftools concat
ALL.chr*.phase3_shapeit2_mvncall_integrated_v5a.20130502.genotypes.vcf.gz
-Oz -o allChromosomes_1000G.vcf.gz
```

3. Extract individuals from specific population groups using PLINK:

```
plink --vcf allChromosomes_100G.vcf.gz --keep Populations_required.txt --make-bed --out
allChromosomes_1000G_PopulationsRequired
```

Population labels for each sample ID in 1000GP phase 3 release can be found at https://www.internationalgenome.org/data-portal/sample.

4. Merge reference sample file and target sample file using steps outlined in Basic Protocol 2, steps 2 to 7.

5. Remove redundant SNPs through LD-pruning. Perform LD-pruning by removing each SNP with $r^2 > 0.1$ within a 50-SNP sliding window (advanced by 10 SNPs at a time) using PLINK:

```
plink --bfile TargetSamples_ReferenceSamples_Merged --indep-pairwise 50 10 0.1
```

Remove `plink.prune.out` file from target and reference merged dataset:

```
plink --bfile TargetSamples_ReferenceSamples_Merged --exclude plink.prune.out --make-bed
  --out TargetSamples_ReferenceSamples_Merged_LD-pruned
```

This will improve the computational efficiency of the ADMIXTURE analysis by reducing the number of SNPs included in the analysis.

6. Determine the correct number of ancestral/source populations contributing to the target population. Implement ADMIXTURE's unsupervised clustering algorithm with CV for a range of K values (e.g., K=3-10), where the number of contributing ancestries/populations is denoted by K:

```
for k in {3..10}; do admixture --cv TargetSamples_ReferenceSamples_Merged_LD-pruned.bed
  ${k} -j4 | tee log${k}; done
```

Inspect the log files to identify the value of K (number of contributing source-populations) with the lowest CV error:

```
grep -h CV log*.out
```

Perform 10 iterations with the correct K value to refine global ancestryproportions:

```
for i in {1..10}; do admixture --cv TargetSamples_ReferenceSamples_Merged_LD-pruned.bed
  K -j4; done
```

Visualize global ancestry proportions for each individual using PONG:

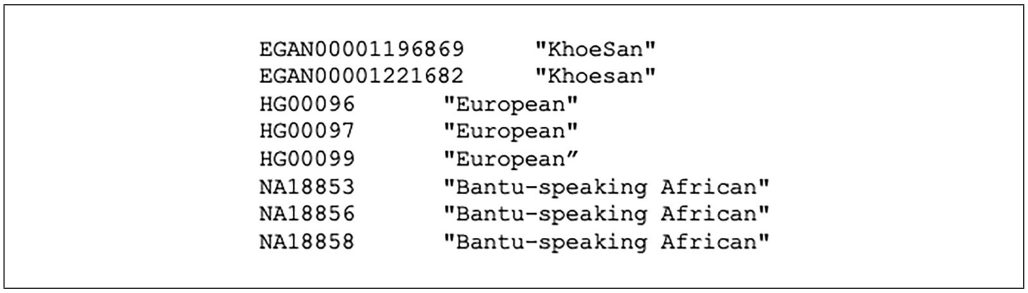
```
pong -m filemap -n pop_order -I ind2pop
```

The following input files are required: pong_filemap, pop_order, and ind2pop. The user documentation for PONG describes the contents and formats of these files in detail (https://github.com/ramachandran-lab/pong/blob/master/README.md).

7. Split the target and reference merged dataset into separate chromosomes before phasing:

```
for i in {1..22}; do plink --bfile TargetSamples_ReferenceSamples_Merged --chr ${i}
    --make-bed --out TargetSamples_ReferenceSamples_Merged.chr${i}; done
```

RFMix requires phased genotype data for ancestry inference. Phasing is the process of inferring haplotypes from genotype data. SHAPEIT (Delaneau et al., 2013) phasing



```
EGAN00001196869     "KhoeSan"
EGAN00001221682     "Khoesan"
HG00096      "European"
HG00097      "European"
HG00099      "European”
NA18853      "Bantu-speaking African"
NA18856      "Bantu-speaking African"
NA18858      "Bantu-speaking African"
```

Figure 3 Example of the sample map file required for RFMix.

software requires recombination maps (which can be downloaded from https://ftp.ncbi.nlm.nih.gov/hapmap/recombination/2011-01_phaseII_B37/).

8. Phase each chromosome separately using SHAPEIT:

```
for i in {1..22}; do shapeit --input-bed TargetSamples_ReferenceSamples_Merged_
chr${i}.bed TargetSamples_ReferenceSamples_Merged_chr${i}.bim TargetSamples_
ReferenceSamples_Merged_chr${i}.fam --input-map genetic_map_GRCh37_chr${i}.txt
--ouput-haps TargetSamples_ReferenceSamples_Merged_chr${i}.haps; done
```

As SHAPEIT was used to pre-phase genotype data prior to imputation (see Basic Protocol 1), the software is used to phase imputed data for consistency.

9. Convert phased files to VCF file format:

```
for i in {1..22}; do shapeit -convert --input-haps TargetSamples_ReferenceSamples_
Merged_chr${i}.haps --output-vcf TargetSamples_ReferenceSamples_Merged_chr${i}.vcf;
done
```

10. Prepare reference and target files required for RFMix, which requires one text file listing the sample IDs for all target individuals and one text file listing the sample IDs for all reference individuals:

```
for i in {1..22}; do vcftools --vcf TargetSamples_ReferenceSamples_Merged_chr${i}.vcf
--keep Target_individuals.txt --recode --out TargetSamples_chr${i}.vcf; done
```

```
for i in {1..22}; do vcftools --vcf TargetSamples_ReferenceSamples_Merged_chr${i}.vcf
--keep Reference_individuals.txt --recode --out ReferenceSamples_chr${i}.vcf; done
```

11. Create the sample map file required for RFMix.

The sample map file is a tab-delimited text file with two columns. The first column contains the sample ID for each individual in the reference sample file. The second column contains the population label for the respective individual. The header of an example of a sample map file is shown in Figure 3.

12. Run RFMix using default parameters:

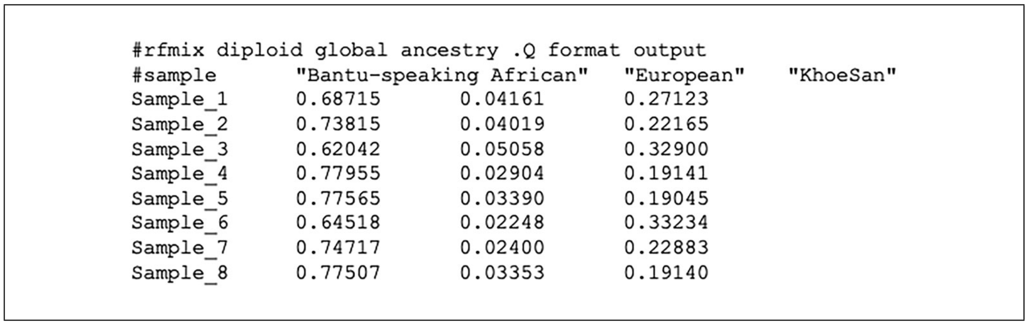
```
for i in {1..22}; rfmix -f TargetSamples_chr${i}.vcf -r ReferenceSamples_chr${i}.vcf -m
Sample_map -g genetic_map_GRCh37_chr${i}.txt --chromosome ${i} --reanalyze-reference
-o RFMix_outputfile_chr${i}; done
```

The `--reanalyze-reference` flag can be implemented to improve accuracy. The number of generations since admixture (if known) can be specified using the `-G` flag.

Among the files output by RFMix (described in more detail here: https://github.com/slowkoni/rfmix/blob/master/MANUAL.md), the ancestry proportions across each chromosome for each individual are listed in the `.Q` files (Fig. 4). Global ancestry proportions can be derived for each individual by calculating the average of each ancestry proportion across all chromosomes.

Sample Data

See the sample map file required for RFMix in Figure 3.



```
#rfmix diploid global ancestry .Q format output
#sample      "Bantu-speaking African"   "European"    "KhoeSan"
Sample_1     0.68715      0.04161      0.27123
Sample_2     0.73815      0.04019      0.22165
Sample_3     0.62042      0.05058      0.32900
Sample_4     0.77955      0.02904      0.19141
Sample_5     0.77565      0.03390      0.19045
Sample_6     0.64518      0.02248      0.33234
Sample_7     0.74717      0.02400      0.22883
Sample_8     0.77507      0.03353      0.19140
```

Figure 4 Example of the global ancestry proportion output (`.Q`) file generated by RFMix.

RFMix outputs global ancestry proportions for each individual in the `.Q` file (Fig. 4).

BASIC PROTOCOL 4

BATCH EFFECT CORRECTION USING PSEUDO-CASE-CONTROL COMPARISONS

Basic Protocol 4 described the pseudo-case-control comparison method for batch effect correction, adapted from prior work (Chen et al., 2022). This method involves coding case/control status by batch followed by running a logistic regression analysis testing each batch against all other batches. For example, the status of all samples from one dataset is coded as a case, whereas the status of every other sample is coded as a control. A logistic regression test is then performed. This procedure is repeated for each batch. If any single dataset has more positive signals compared to the other datasets, then batch effects may be responsible for producing spurious results. If batch effects are present, the genomic inflation factor (λ) for the pseudo-case-control comparisons will be greater than one. Batch effects can be resolved by removing those SNPs that pass the threshold for significant from the merged dataset, as these SNPs are affected by batch effects. The standard genome-wide threshold for significance (5×10^{-8}) may be overly stringent for samples obtained from admixed populations. The R package *STEAM* (Significance Threshold Estimation for Admixture Mapping) (Grinde et al., 2019) calculates a less-stringent significance threshold for analyses using admixed population, taking the global ancestry proportions and number of generations since admixture into account.

Necessary Resources

Hardware

A computer with as much memory (≥8 GB) and computing power as possible or access to an HPC cluster. A computer running either a 64-bit Linux-based distribution (e.g., Ubuntu) or macOS is required.

Software

PLINK v2.0 (*https://www.cog-genomics.org/plink2/*)
R v4.3.1 (*https://www.r-project.org*)

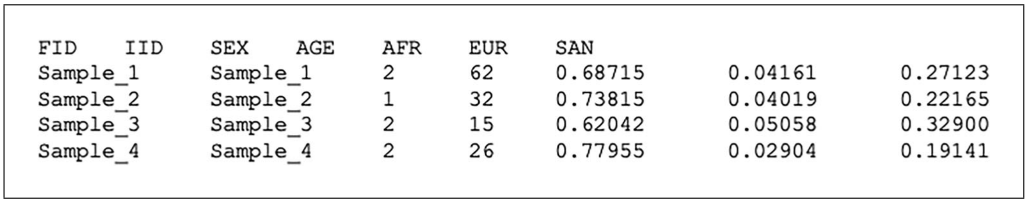
Files

Merged target PLINK binary files (`Merged_files_QC`)
Covariate file (Fig. 5)

1. Create a covariate file for the merged dataset.

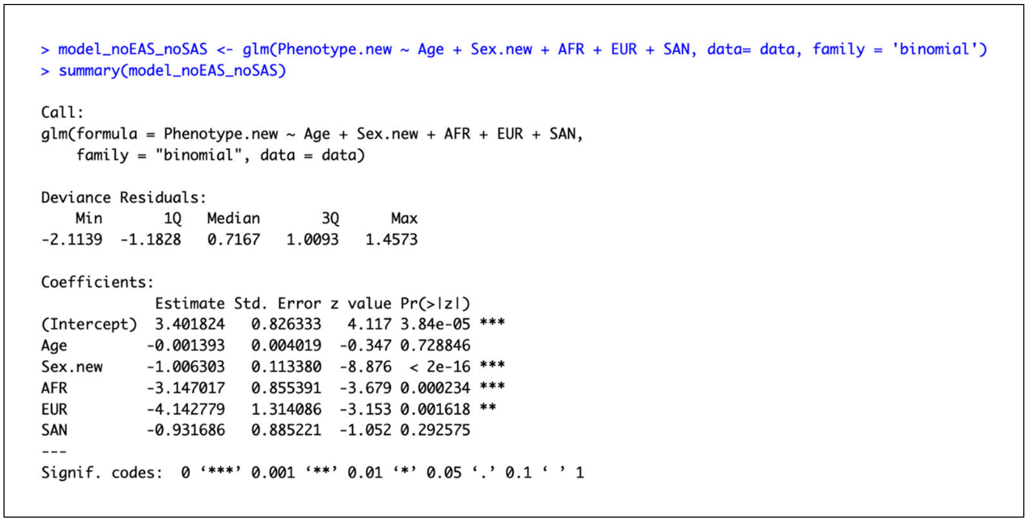
   This is a tab-delimited text file with sample ID, phenotype and sex information, age, and global ancestry proportions (determined by ADMIXTURE or RFMix) for each individual in the merged file (Fig. 5).

2. Determine the covariates that have the greatest effect on the outcome, which can be done by running a logistic regression test in R using the covariate file:



```
FID    IID    SEX    AGE    AFR    EUR    SAN
Sample_1      Sample_1      2      62     0.68715     0.04161     0.27123
Sample_2      Sample_2      1      32     0.73815     0.04019     0.22165
Sample_3      Sample_3      2      15     0.62042     0.05058     0.32900
Sample_4      Sample_4      2      26     0.77955     0.02904     0.19141
```

Figure 5 Example of a covariate file required for Basic Protocol 4.



```
> model_noEAS_noSAS <- glm(Phenotype.new ~ Age + Sex.new + AFR + EUR + SAN, data= data, family = 'binomial')
> summary(model_noEAS_noSAS)

Call:
glm(formula = Phenotype.new ~ Age + Sex.new + AFR + EUR + SAN,
    family = "binomial", data = data)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.1139  -1.1828   0.7167   1.0093   1.4573

Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)  3.401824   0.826333   4.117 3.84e-05 ***
Age         -0.001393   0.004019  -0.347 0.728846
Sex.new     -1.006303   0.113380  -8.876  < 2e-16 ***
AFR         -3.147017   0.855391  -3.679 0.000234 ***
EUR         -4.142779   1.314086  -3.153 0.001618 **
SAN         -0.931686   0.885221  -1.052 0.292575
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Figure 6 Example of the output of the logistic regression test to determine which covariates have the greatest effect on the outcome.

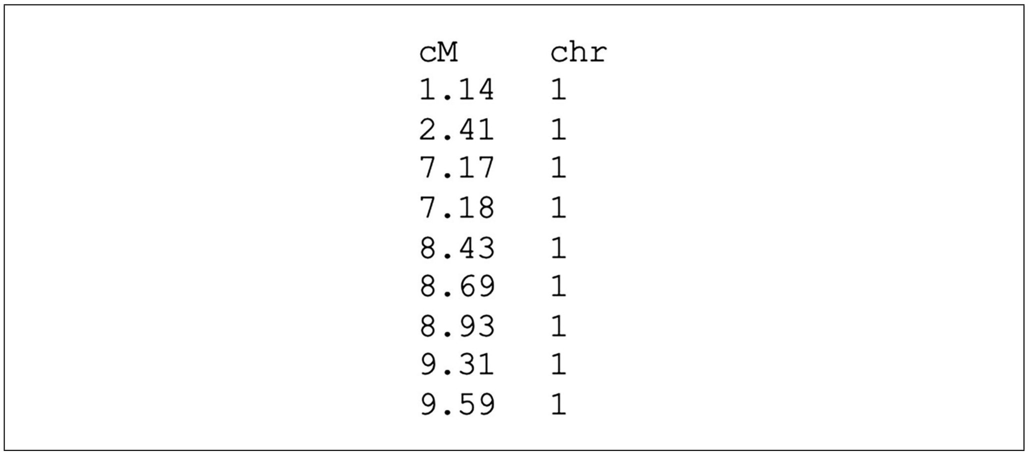
```
R
## Model
## Read data
data <- read.table("Covariates_data.txt")
View(data)
colnames(data) <- c("FID", "IID", "Phenotype", "Sex", "Age", "AFR", "EAS", "EUR", "SAN",
   "SAS")
# Recode Phenotype (control=0, case=1)
data$Phenotype.new <- ifelse(test = data$Phenotype ==1, yes=0, no=1)
# Recode Sex (male=0, female=1)
data$Sex.new <- ifelse(test =data$Sex==1, yes=0, no=1)
## Run logistic model
model <- glm(Phenotype.new ~ Age + Sex.new + AFR + EUR + SAN, data= data, family =
   ‘binomial’)
summary(model)
```

Select the covariates that have significant Z-scores (Fig. 6).

These covariates will be run in the pseudo-case-control comparison logistic regression test.

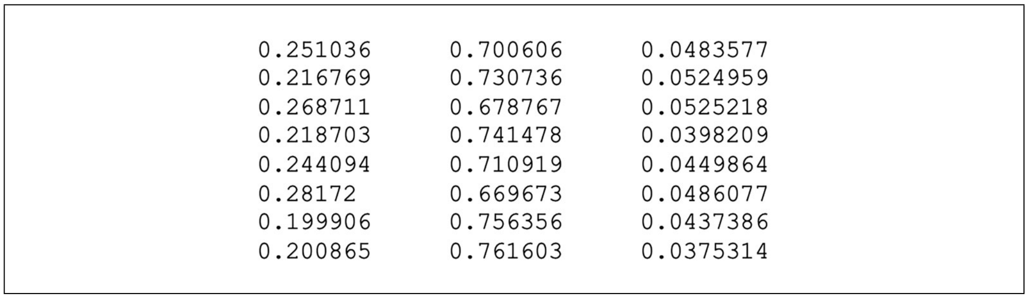
3. Because the standard genome-wide threshold for significance (5×10^{-8}) might be overly stringent for samples obtained from admixed populations, calculate a new genome-wide threshold, which takes global ancestry proportions into account. Determine the genome-wide significance threshold value using *STEAM*.

STEAM requires a `map` *file containing the chromosome number and genetic position (in centimorgans) of each marker being tested. This information can be extracted from the first and fourth columns of the* `.msp.tsv` *files generated by RFMix in Basic Protocol 3 (Fig. 7). STEAM also requires the admixture proportions generated by RFMix (Fig. 8). A total of 10,000 iterations of the calculation are performed to generate an accurate result.*



```
cM     chr
1.14   1
2.41   1
7.17   1
7.18   1
8.43   1
8.69   1
8.93   1
9.31   1
9.59   1
```

Figure 7 Example of the map file required for *STEAM*.



```
0.251036     0.700606     0.0483577
0.216769     0.730736     0.0524959
0.268711     0.678767     0.0525218
0.218703     0.741478     0.0398209
0.244094     0.710919     0.0449864
0.28172      0.669673     0.0486077
0.199906     0.756356     0.0437386
0.200865     0.761603     0.0375314
```

Figure 8 Example of the admixture proportion file required for *STEAM*.

```
R
## Install and load STEAM package
library(devtools)
install_github("kegrinde/STEAM")
library(STEAM)
## Read data
p <- read.table(“Admixture_propostions.txt”)
m <- read.table(“Map_file.txt”)
## Calculate new genome-wide significance threshold
get_thresh_simstat(g = 15, map = m, props = p, nreps = 10000)
```

4. Improve the stability of the logistic regression test by removing SNPs with MAF < 1%:

```
plink --bfile Merged_files_QC --maf 0.001 --make-bed --out Merged_files_QC_maf
```

5. Code one batch/dataset as case (2) in the `.fam` file. Code all other batches/datasets as controls (1).

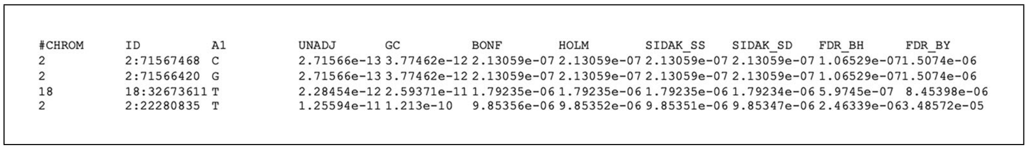
6. Run logistic regression model (use sex, age, and global ancestry proportion covariates) using PLINK:

```
plink --bfile Batch1_vs_others --glm sex --covar Covariates.txt --covar-name AGE,AFR,
EUR,SAN --covar-variance-standardize --adjust --ci 0.95 --out Batch1_vs_others
```

7. Inspect the `Batch1_vs_others.PHENO1.glm.logistic.hybrid.adjusted` output file (Fig. 9). Create a list of SNPs with Benjamini-Hochberg *p*-values less than the significance value calculated by *STEAM*.

8. Repeat steps 4 to 7 for each batch/dataset.

9. Exclude the list of SNPs affected by batch from the merged dataset:



```
#CHROM     ID          A1        UNADJ       GC          BONF        HOLM        SIDAK_SS    SIDAK_SD    FDR_BH      FDR_BY
2          2:71567468  C         2.71566e-13 3.77462e-12 2.13059e-07 2.13059e-07 2.13059e-07 2.13059e-07 1.06529e-071.5074e-06
2          2:71566420  G         2.71566e-13 3.77462e-12 2.13059e-07 2.13059e-07 2.13059e-07 2.13059e-07 1.06529e-071.5074e-06
18         18:32673611 T         2.28454e-12 2.59371e-11 1.79235e-06 1.79235e-06 1.79235e-06 1.79234e-06 5.9745e-07  8.45398e-06
2          2:22280835  T         1.25594e-11 1.213e-10   9.85356e-06 9.85352e-06 9.85351e-06 9.85347e-06 2.46339e-063.48572e-05
```

Figure 9 Example of the logistic regression output file with adjusted *p*-values generated by PLINK.

```
plink --bfile Merged_files_QC --extract BatchEffectedSnps.txt --make-bed --out
   Merged_files_QC_BatchEffectCorrected
```

Sample Data

Sample data include a header of an example of a covariate file required for Basic Protocol 4 (Fig. 5), the results of a logistic regression test to determine which covariates have the greatest effect on the outcome (Fig. 6), a header map file generated from columns 1 and 4 of `.msp.tsv` RFMix output file (Fig. 7), the header of the admixture proportion file required for running *STEAM* (Fig. 8), and the header of the logistic regression output file with adjusted p-values generated by PLINK (Fig. 9).

Guidelines for Understanding Results

A logistic regression association test can be performed to determine if the data harmonization procedures were successful. Quantile-quantile (QQ) plots can be constructed to quantify the degree of inflation and determine if residual technical artifacts are confounding results.

COMMENTARY

Background Information

Data harmonization is a feasible strategy to increase sample sizes and improve the power of GWASs and other association tests. We have applied these protocols to merge separate genotype datasets without introducing spurious associations caused by batch effects or other technical artifacts. We combined six case-control genome-wide datasets [comprising five-way admixed South African Colored (SAC) individuals] to investigate host genetic variants associated with tuberculosis (TB) susceptibility. These datasets had been individually analyzed with little success, and we hypothesized that by combining these datasets, we could identify novel TB host genetic associations or confirm known associations. We applied our data harmonization guidelines for merging genotype data obtained from admixed individuals to produce a harmonized, high-quality dataset comprising 1544 individuals (952 TB cases and 592 healthy controls) free from technical artifacts. We then applied the local ancestry adjusted allelic association (LAAA) model (Duan et al., 2018) to identify ancestry-specific genetic variants associated with TB susceptibility. One SNP (*rs74828248*), located on chromosome 20q13.33, was significantly associated with TB susceptibility while adjusting for Bantu-speaking African local ancestry (*p*-value = 2.272 × 10-6, OR = 0.316, SE = 0.244). A suggestive association peak in the *HLA-II* region was also identified using the LAAA model while adjusting for KhoeSan ancestry. This association signal has previously been observed in a TB meta-analysis conducted by the International Tuberculosis Host Genetics Consortium (Schurz et al., 2022) and in an independent study conducted in a South African cohort (Chihab et al., 2023). Moreover, we achieved greater power to identify statistically significant markers using our harmonized dataset compared to previous analyses. Given our sample size of 1544 participants, we had a 95% chance to correctly reject the null hypothesis for markers with effect sizes from 0.2. A previous study performed by Swart et al. (2021) had a 95% chance to correctly reject the null hypothesis for large (>0.5) and medium (>0.3) effect sizes using a sample size of 735 participants from the same population group. Hence, our data harmonization guidelines have been applied to real biological data and have been shown to improve the power of downstream analyses without confounding results.

Data harmonization is necessary for large consortia that recruit thousands of samples over a period of time and require several different laboratories and facilities for genotyping and data processing. Additionally, these procedures can be employed by smaller projects to leverage external controls from public databases and make use of valuable data that may otherwise have been excluded from the analyses. However, data harmonization strategies are not widely adopted due to the challenges that arise from merging data. Guidelines for these strategies are scarce and often conflicting. For example, PCA is frequently recommended as a batch effect correction strategy despite its limited capacity to distinguish batch effects from population structure (Leek et al., 2010; Nyamundanda et al., 2017; Reese et al., 2013). Complicated procedures like data harmonization should have well-documented, robust guidelines for reproducible results. As part of this article, we present the first guidelines for merging genome-wide genotype data obtained from multi-way admixed populations. Our data harmonization strategy involves the following four steps:

Processing individual datasets (Basic Protocol 1)

Individual datasets should be processed separately at first, using standard QC procedures in which samples with high rates of missingness and variants with low genotype call rates are removed. Datasets are QC'd and imputed separately to achieve the greatest number of intersecting sites with high imputation quality across all datasets. Following QC, individual datasets should be phased and imputed separately. Poorly imputed sites should be removed to limit uncertain genotypes in downstream analyses, which could cause spurious associations.

Merging separate datasets (Basic Protocol 2)

Following post-imputation QC, separate datasets can be merged. Only sites common across all datasets should be merged. This will facilitate the merging process and ensure markers included in downstream analyses are consistent across all datasets, thereby limiting the potential for spurious associations. Additional QC procedures can be applied after merging, such as the removal of related individuals.

Ancestry inference (Basic Protocol 3)

Global ancestry proportions should be estimated using ancestry deconvolution software (like ADMIXTURE or RFMix). Global ancestry proportions should be included in null GWASs when correcting for population stratification and to account for the variation in ancestral contributions across all admixed individuals.

Batch effect correction (Basic Protocol 4)

The pseudo-case-control comparison method for batch effect correction proposed by Chen et al. (2022) can effectively be applied to admixed individuals. However, we recommend the inclusion of global ancestry proportions as covariates in place of PCA to account for differences in population structure among individuals.

These data harmonization guidelines employ thorough QC procedures to remove technical artifacts as well as uncertain genotypes following imputation and correct for the presence of batch effects in a merged dataset. Because multiple genotyping arrays are available for use in human genetic studies and new arrays are continuously being developed, data harmonization procedures that make use of imputation are essential to allow merging of disparate genotype arrays. This harmonization procedure for merging genotype data from multiple sources can be employed for other applications where individual-level genotype information is required.

Although packaged software pipelines are appealing to researchers for efficient workflows, we have chosen to not package these guidelines into a software program. Admixed populations are highly diverse with respect to their contributing ancestries. Hence, we believe that being able to tailor the guidelines to a unique population group is an advantage of an unpackaged set of guidelines, in which the researcher can adapt the thresholds and reference panels to suit their population under study.

Critical Parameters

Factors that influence the protocols and to which special attention should be paid are as follows:

Imputation reference panel (Basic Protocol 1)

As haplotype structures across different populations vary and imputation accuracy is dependent on the adequate matching of individual haplotypes to a reference, it is important to use haplotype reference panels that are matched to the population under study (Schurz et al., 2019; Sengupta et al., 2023).

Reference samples included for ancestry inference (Basic Protocol 3)

Similarly, ancestry inference accuracy is dependent on the adequate matching of target samples to reference individuals with similar

Table 1 Troubleshooting Guide for Genomic Data Harmonization

Problem	Solution
Merging datasets—multiple incongruent sites	Ensure all datasets are aligned to the same assembly of the reference human genome. Datasets aligned to different versions can be lifted over using the GATK LiftoverVCF function (*https://gatk.broadinstitute.org/hc/en-us/articles/360037060932-LiftoverVcf-Picard-*).
Imputation—large proportion of low-quality genotypes	Remove sites with low INFO scores (e.g., INFO scores < 0.8) and redo the imputation procedure. The second round of imputation corrects poorly imputed genotypes, and subsequent QC of the re-imputed data should retain more high-quality sites.
ADMIXTURE—running groups	ADMIXTURE performs best when approximately equal numbers of reference populations and admixed populations are included. Thus, larger target datasets may need to be divided into smaller running groups with individuals from reference populations. Steps 5 and 6 from Basic Protocol 3 must be performed on each running group.
Phasing and RFMix—phase switching	RFMix implements a phase-correction algorithm that can cause phase switching. This is a known issue with RFMix with no straightforward solution: *https://github.com/slowkoni/rfmix/issues/7*.
GWAS—inflated results following Basic Protocol 4	Basic Protocol 4 can be repeated using a more stringent genome-wide significance threshold if required.

Table 2 Alternative Software

Software	Use	Reference
REAP (Relatedness Estimation in Admixed Populations)	Relationship inference for admixed populations	Thornton et al., 2012
RelateAdmix	Relationship inference for admixed populations	Conomos et al., 2016
STRUCTURE	Global ancestry inference	Pritchard et al., 2000

haplotype structure. Thus, it is important to use reference samples that are obtained from population groups that are either similar to or at least proxies of the ancestral populations of the target samples under study. Appropriate reference populations can be selected based on the demographic history of the population under study.

Troubleshooting

Some potential problems and their respective solutions are presented in Table 1.

Software alternatives

Although our protocols detail steps for data harmonization using specific software, we acknowledge that researchers may wish to use other programs to achieve the same task based on their preferences and previous experience with using specific software. Alternative software for calculating relatedness and inferring ancestral proportions, which may be used to accomplish what is done in the above protocols, is presented in Table 2.

Author Contributions

Dayna Croock: Formal analysis; investigation; methodology; writing—original draft; writing—review and editing. **Yolandi Swart**: Formal analysis; methodology; supervision; writing—review and editing. **Haiko Schurz**: Conceptualization; methodology; supervision; writing—review and editing. **Desiree C. Petersen**: Conceptualization; project administration; supervision; writing—review and editing. **Marlo Möller**: Conceptualization; data curation; project administration; resources; supervision; writing—review and editing. **Caitlin Uren**: Conceptualization; data curation; project administration; supervision; writing—review and editing.

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Conflict of Interest

There are no conflicts of interest to declare.

Data Availability Statement

No new genetic data were generated for this study. However, summary statistics for the quality and accuracy assessment of the genetic data will be made available to researchers who meet the criteria for access after application to the Health Research Ethics Committee (HREC) of Stellenbosch University. Requests to access these datasets should be directed to Marlo Möller, marlom@sun.ac.za.

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